

STUDIES ON SYMPATHOMIMETIC AMINES. I. THE BIOTRANSFORMATION AND PHYSIOLOGICAL DISPOSITION OF *L*-EPHEDRINE AND *L*-NOREPHEDRINE¹

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Numerous *in vitro* studies have implicated monoamine oxidase, phenol oxidase, and cytochrome oxidase in the biotransformation of various sympathomimetic amines (Beyer, 1946). However, the part that these enzymes play in the intact animal is conjectural without definitive information concerning the chemical fate of the sympathomimetic amines in the body.

The present communication, the first of a series of papers on the fate of sympathomimetic amines in the body, is concerned with the metabolic fate and physiological disposition of *L*-ephedrine and its transformation products in a number of animal species. It will be shown that ephedrine is transformed along two metabolic pathways, one involving demethylation and the other hydroxylation to yield metabolic products which possess pressor activity and that various animal species show considerable differences in the relative importance of the two metabolic routes.

CHEMICAL METHODS. Estimation of ephedrine and norephedrine: Both ephedrine and norephedrine can be quantitatively extracted into benzene and assayed by the methyl orange reaction (Brodie and Udenfriend, 1945). The two drugs are determined in the presence of each other from calculations based on their partition ratios between petroleum ether and aqueous alkali. In the determination, one sample of biological material is extracted with benzene and the total methyl orange reacting material assayed. In another sample the distribution ratio of the methyl orange reacting material between 1 volume of aqueous alkali and 3 volumes of petroleum ether is determined. The distribution ratio of the methyl orange reacting material in the unknown sample is a function of the relative concentrations of ephedrine and norephedrine, since petroleum ether extracts about 90 per cent of the ephedrine and 30 per cent of the norephedrine. The concentration of ephedrine and norephedrine in the biological material is calculated by a simple algebraic equation described below.

Procedure for plasma and urine: Pipet 15 ml. of plasma or diluted urine (containing 10 to 100 microgm. of ephedrine plus norephedrine) into a 50 ml. graduated tube containing about 7 gm. of solid NaCl. Add 1 ml. of 10 *N* NaOH, dilute to 20 ml. with water and mix thoroughly (solution A).

Total methyl orange reacting material (ephedrine plus norephedrine) is determined as follows: Transfer 4 ml. of solution A to a 35 ml. glass-stoppered centrifuge tube containing 10 ml. of benzene² and shake for 10 minutes. Centrifuge the tube and transfer 8 ml. of the benzene phase to a 15 ml. glass-stoppered centrifuge tube containing 0.5 ml. of isoamyl

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² Solvents are purified by successive washings with 1 *N* NaOH, 1 *N* HCl, and 2 washings with water.

alcohol.² Add 0.6 ml. of methyl orange reagent³ and shake vigorously for about 5 minutes. Centrifuge the tube and transfer 6 ml. of the supernatant benzene phase to a cuvet containing 1 ml. of a solution of 2 per cent by volume of sulphuric acid in absolute ethanol. Work as rapidly as possible to minimize the adsorption of the methyl orange complex on the sides of the tube. Determine the optical density (*T*) in a spectrophotometer at 540 mμ. A reagent blank carried through the above procedure is used for the zero setting.

The distribution ratio of the methyl orange reacting material between aqueous alkali and petroleum ether is determined as follows: Transfer 5 ml. of solution A to a 60 ml. glass-stoppered bottle containing 15 ml. of petroleum ether. Shake for 10 minutes and centrifuge the bottle. Transfer 4 ml. of the aqueous phase to a 35 ml. centrifuge tube containing 10 ml. of benzene, and determine the residual methyl orange reacting material (optical density *D*) as described above.

Standard solutions of ephedrine and norephedrine are prepared by handling known amounts of each drug in the same manner as the unknown. Standards are run concurrently with the unknowns, since there is a small daily variation in the distribution ratios of ephedrine and norephedrine between aqueous alkali and petroleum ether. Optical densities of 0.220 and 0.190 are obtained (Coleman Model 6 spectrophotometer) when 10 microgm. of ephedrine and norephedrine, respectively, in 10 ml. of benzene react with methyl orange.

Procedure for tissues: Emulsify about 10 gm. of tissue with 40 ml. of 0.1 *N* HCl in an electrically driven homogenizer consisting of a glass cylindrical cup in which a close fitting ground glass pestle is mechanically rotated.⁴ Transfer 20 ml. of the tissue homogenate to a 50 ml. centrifuge tube and precipitate proteins by adding 10 ml. of a 20 per cent trichloroacetic acid solution. Centrifuge the tube and transfer a 15 ml. aliquot of the supernatant solution to a 50 ml. centrifuge tube containing about 7 gm. of solid NaCl. Add 1.5 ml. 10 *N* NaOH, dilute to 20 ml. with water, and stir thoroughly. Proceed as described for plasma and urine for the estimation of ephedrine and norephedrine.

Ephedrine and norephedrine added to biological material were recovered with adequate precision (95 ± 5 per cent). Recoveries are less precise when the ratio of one compound to the other exceeds five.

The ephedrine and norephedrine concentrations in the biological material are calculated from the following equation:

$$\frac{D - \frac{N_D}{N_T} T}{\frac{E_D}{E_T} - \frac{N_D}{N_T}} = E \text{ (optical density of ephedrine in the unknown solution)}$$

T = optical density of ephedrine plus norephedrine in the unknown solution

D = optical density of ephedrine plus norephedrine in the unknown solution after shaking with petroleum ether

$\frac{N_D}{N_T}$ = distribution ratio of norephedrine between aqueous alkali and petroleum ether

where *N_D* = optical density of standard norephedrine solution after shaking with petroleum ether and *N_T* = optical density of standard norephedrine solution

$\frac{E_D}{E_T}$ = distribution ratio of ephedrine between aqueous alkali and petroleum ether

² A stock solution of methyl orange reagent is made by dissolving 500 mgm. of methyl orange in 100 ml. of warm water. The methyl orange solution is washed several times with equal volumes of chloroform. An aliquot of the resulting solution is diluted with an equal volume of saturated boric acid solution immediately before use.

⁴ A glass homogenizer can be obtained from Scientific Glassware Co., Bloomfield, N. J.

where E_D = optical density of standard ephedrine after shaking with petroleum ether and E_T = optical density of standard ephedrine solution

$T - E = N$ (optical density of norephedrine in the unknown solution).

Estimation of p-hydroxyephedrine plus p-hydroxynorephedrine: In the following procedure *p*-hydroxyephedrine and *p*-hydroxynorephedrine are not separated from each other but are isolated together from biological material by extraction into isoamyl alcohol at pH 9-10. The extraction is augmented by saturating the aqueous phase with sodium chloride. The phenols are returned to an aqueous phase by the addition of petroleum ether to the isoamyl alcohol solution and shaking with 0.1 *N* HCl. The aqueous solution is adjusted with NH_4OH to pH 9 to 10, and reacted with 4-aminoantipyrine and potassium ferricyanide. The resulting indophenols are assayed spectrophotometrically at 500 $\text{m}\mu$.

Procedure: Pipet 5 ml. of biological material into a 60 ml. glass-stoppered bottle containing 2 to 3 gm. solid NaCl. Adjust the pH to 9-10 by the addition of solid Na_2CO_3 . Add 25 ml. of isoamyl alcohol² and shake for 10 minutes. Centrifuge the bottle and transfer a 20 ml. of aliquot of the isoamyl alcohol to another 60 ml. bottle containing 20 ml. petroleum ether and 5 ml. 0.1 *N* HCl. Shake for 5 minutes and transfer 4 ml. of the acid layer to a cuvet containing 1 ml. 1 *N* NH_4OH . (The pH should now be between 9-10.) Add 1 ml. 0.8 per cent potassium ferricyanide followed by 1 ml. of 0.25 per cent 4-aminoantipyrine, allow to stand for 5 minutes, and read the optical density at 500 $\text{m}\mu$ in a spectrophotometer. A blank containing 4-aminoantipyrine, potassium ferricyanide and NH_4OH is used to set the spectrophotometer to read 100 per cent transmission.

The distribution of *p*-hydroxyephedrine and *p*-hydroxynorephedrine in an isoamyl alcohol-salt saturated water system is such that with volumes of 25 and 5 ml., respectively, about 90 per cent of the phenols are extracted in the organic phase. Standards are, therefore, prepared by handling known amounts of either *p*-hydroxyephedrine or *p*-hydroxynorephedrine in the same manner as the unknown solution. *p*-Hydroxyephedrine and *p*-hydroxynorephedrine added to biological material in amounts from 10 to 200 microgm. were recovered with satisfactory precision (95 ± 8 per cent).

EXPERIMENTAL. *Isolation and identification of ephedrine and norephedrine in the urine of dogs:* Two dogs received 50 mgm. per kgm. of *l*-ephedrine hydrochloride intraperitoneally and the urine was collected for the subsequent 24 hours. An aliquot of the pooled urine was made alkaline with NaOH and extracted with three volumes of chloroform. The extract contained considerable amounts of basic material which gave a methyl orange reaction (Brodie and Udenfriend, 1945). The chloroform extract was reduced to a small volume at room temperature under a stream of nitrogen. The material reacting with methyl orange was subjected to a 99 transfer counter-current distribution, using equal volumes of pH 8.7 borate buffer (0.2 *M*) and chloroform. Under these conditions ephedrine has a distribution ratio of about 1. After the counter-current distribution, the material reacting with methyl orange was determined in each tube. The total optical density of the methyl-orange reacting material in each tube was plotted against the serial number of the tube. The distribution curve indicated that two compounds reacting with methyl orange were present (fig. 1). The contents of tubes 32-50, which contained material with partition ratios similar to that of ephedrine, were pooled. The combined aqueous phase was made alkaline and the total basic material brought into the combined organic phase by shaking. The chloroform phase was evaporated to dryness at room temperature under a stream of nitrogen, and the residue was dissolved in anhydrous ether. Dry

gaseous HCl was passed through the ether and the resulting precipitate was recrystallized twice from an alcohol-ether mixture, yielding crystals which melted sharply at 220°C. (uncorr.). An authentic sample of *l*-ephedrine hydrochloride melted at 219°C. (uncorr.) and a mixture of the two compounds showed no depression of the melting point. The ultraviolet absorption spectra of the apparent and authentic *l*-ephedrine hydrochloride in 0.1 *N* HCl and 0.1 *N* NaOH were found to be almost superimposable. Conclusive evidence that the substance isolated from urine was *l*-ephedrine was obtained by comparison of infra-red spectra of the isolated and authentic *l*-ephedrine hydrochloride, which were identical.

The material in tubes 58-72, isolated in the same manner as above, yielded a crystalline material with a melting point of 175-176°C (uncorr.). An authentic sample of *l*-norephedrine hydrochloride melted at 175°C. (uncorr.) and a mixed

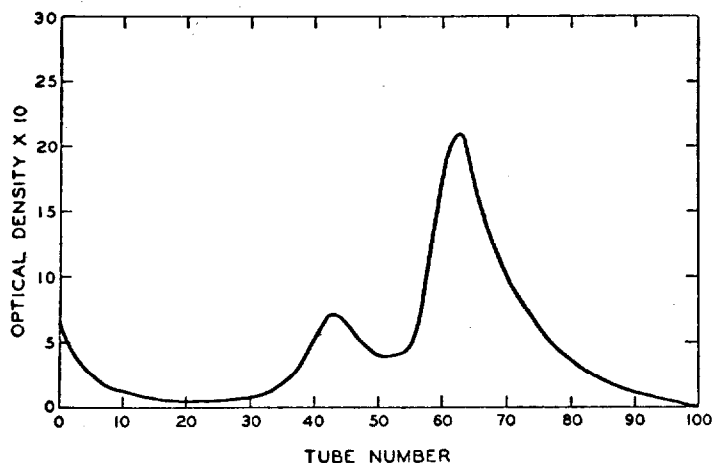


FIG. 1. Counter-current distribution of methyl orange reacting material isolated from the urine of two dogs that received 50 mgm. per kgm. of *l*-ephedrine hydrochloride.

System 0.2 *M* borate buffer pH 8.7, and chloroform (equal volumes).

melting point of the two compounds showed no depression. The ultraviolet absorption spectra of the isolated metabolite and of authentic *l*-norephedrine in 1 *N* HCl and 0.1 *N* NaOH were found to be almost superimposable. Apparent *l*-norephedrine hydrochloride isolated from the urine gave the following per cent composition on analysis:

Calculated: for $C_9H_{11}ONCl$: C, 57.59; H, 7.52

Found: C, 57.25; H, 7.41

Conclusive evidence that the substance isolated from the urine was *l*-norephedrine was obtained by comparison of infra-red spectra of the isolated and authentic *l*-norephedrine hydrochloride, which were identical.

Isolation and identification of p-hydroxyephedrine and p-hydroxynorephedrine in the urine of dogs: An aliquot of the pooled urine was saturated with solid sodium chloride, adjusted to pH 9-10 with solid Na_2CO_3 , and extracted three times with

equal volumes of isoamyl alcohol. The isoamyl extract was reduced to a 15 ml. volume *in vacuo*. Phenolic material was returned to an aqueous phase by adding two volumes of petroleum ether to the isoamyl alcohol extract and shaking with 10 ml. of 0.1 *N* HCl. The acid extract was adjusted to pH 10.3 with solid Na_2HPO_4 and NaOH (1 *N*). The phenolic material was subjected to a 24 transfer counter-current distribution, using equal volumes of pH 10.3 borate buffer (0.2 *M*), and isoamyl alcohol. Under these conditions the phenolic material, as estimated by the 4-aminoantipyrine reaction (see Chemical Methods) was distributed about equally between each phase. After the counter-current distribu-

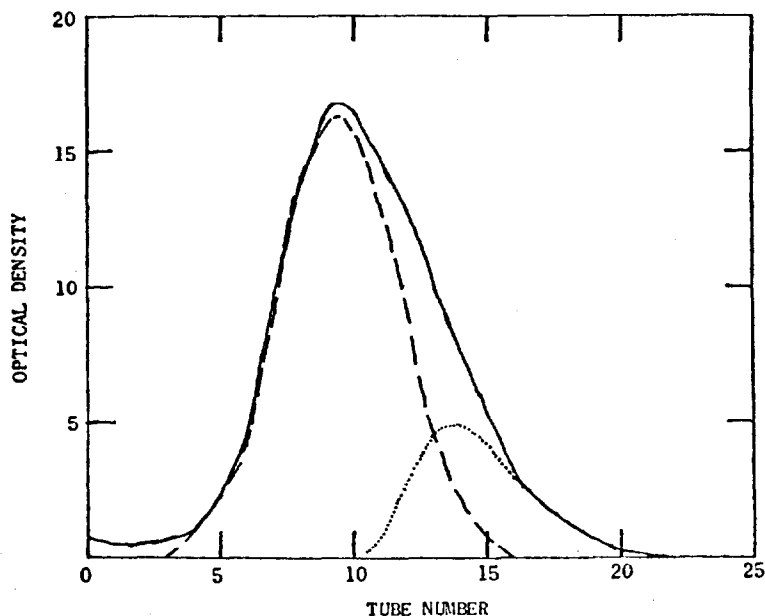


FIG. 2. Counter-current distribution of phenolic compounds isolated from the urine of two dogs that received 50 mgm. per kgm. of *l*-ephedrine hydrochloride.

System 0.2 *M* borate buffer pH 10.3, isoamyl alcohol (equal volumes). Experimental distribution (solid line), theoretical distribution for *p*-hydroxynorephedrine (dashed line), experimental distribution minus theoretical distribution (dotted line).

tion, the phenolic material was determined in each tube. The total optical density of the phenolic material in each tube was plotted against the serial number of the tube. The distribution curve showed the presence of more than one phenolic product (fig. 2). Tubes 4–10 contained material with partition coefficients similar to that of authentic *p*-hydroxynorephedrine. The theoretical distribution curve of *p*-hydroxynorephedrine was calculated from its experimentally established partition ratio. Subtracting the calculated curve from the observed curve showed the presence of relatively small amounts of a second phenolic compound (tubes 11–20). The material in tubes 15–19 had approximately the same partition ratio as that of *p*-hydroxyephedrine. Further evidence for the identity of the phenols

was obtained by the technic of comparative distribution ratios (Brodie *et al.*, 1947). Distribution ratios of the phenolic compounds in tubes 4-10 (apparent *p*-hydroxynorephedrine) and tubes 15-19 (apparent *p*-hydroxyephedrine) between organic solvents and buffers at various pH values were compared with those of authentic samples (table 1). The results confirmed the presence of *p*-hydroxynorephedrine and *p*-hydroxyephedrine.

The urine was also examined for the presence of 3,4-dihydroxynorephedrine, a potent pressor agent, by the method of Beyer and Shapiro (1945). No detectable amounts of this compound were found.

The amounts of ephedrine and its transformation products excreted in urine of dogs: The urine of five dogs, each of which had received 50 mgm. per kgm. of *l*-ephedrine hydrochloride intraperitoneally was analyzed for ephedrine and its transformation products. The urine had been collected for 24 hours following the drug administration; negligible amounts of ephedrine or its transformation

TABLE 1

The distribution of apparent hydroxynorephedrine and hydroxyephedrine and the authentic substances between organic solvents and water at various pH values

The fraction of the compounds extracted at various pH values is expressed as the ratio of the amount of compound in the organic phase to total compound.

pH	SOLVENT	AUTHENTIC HYDROXY- NOREPHEDRINE	APPARENT HYDROXY- NOREPHEDRINE (TUBES 4-10)	AUTHENTIC HYDROXY- EPHEDRINE	APPARENT HYDROXY- EPHEDRINE (TUBES 15-19)
6.0	Isoamyl Alcohol	0.18	0.18	—	—
7.4	Isoamyl Alcohol	0.34	0.35	—	—
8.2	Isoamyl Alcohol	0.75	0.75	—	—
9.6	Isoamyl Alcohol	0.80	0.81	—	—
10.3	Isoamyl Alcohol	0.72	0.73	0.88	0.88
11.4	Isoamyl Alcohol	0.26	0.27	0.51	0.53
10.3	Ether	0.08	0.10	0.26	0.25

products were excreted after this time. About 6 per cent of the administered ephedrine was excreted as the unchanged drug, about 58 per cent as norephedrine, and about 1.5 per cent as *p*-hydroxyephedrine plus *p*-hydroxynorephedrine (table 2).

Plasma levels of ephedrine and norephedrine after the administration of ephedrine to dogs: Plasma concentrations of ephedrine and norephedrine were measured at various time intervals following the intravenous administration of 20 mgm. per kgm. of *l*-ephedrine hydrochloride (fig. 3, typical of four experiments). Plasma levels of ephedrine declined about 50 per cent per hour, whereas those of its derived product, norephedrine, rose to considerable levels, indicating that ephedrine was rapidly demethylated. The levels of norephedrine persisted long beyond the time when those of ephedrine had declined to negligible proportions.

The distribution of ephedrine and norephedrine in tissues: The extent to which

* Ephedrine and norephedrine values in this report are expressed in terms of the free base.

ephedrine and norephedrine were bound to plasma proteins was examined by dialysis against isotonic phosphate buffer of pH 7.4 at 37°C. for 20 hours. Visking membranes were used as dialysis bags. At plasma concentrations of 10 mgm. per liter approximately 12 per cent of ephedrine and 20 per cent of norephedrine were found to be bound to the nondiffusible constituents of the proteins.

TABLE 2

The metabolic fate of ephedrine in the dog

Recovery of ephedrine and its metabolic products from the urine of dog given 50 mgm./kgm. of *l*-ephedrine intraperitoneally

The urine was collected over a period of 24 hours. The proportions of the various metabolites in the urine are expressed in percentage of the amount of ephedrine administered.

DOG NO.	EPHEDRINE	NOREPHEDRINE	TOTAL <i>p</i> -HYDROXYEPHEDRINE AND <i>p</i> -HYDROXYNOREPHEDRINE
	%	%	%
1	4.2	58	1.2
2	2.6	65	1.6
3	2.1	50	1.6
4	4.1	63	1.9
5	20.0	53	—

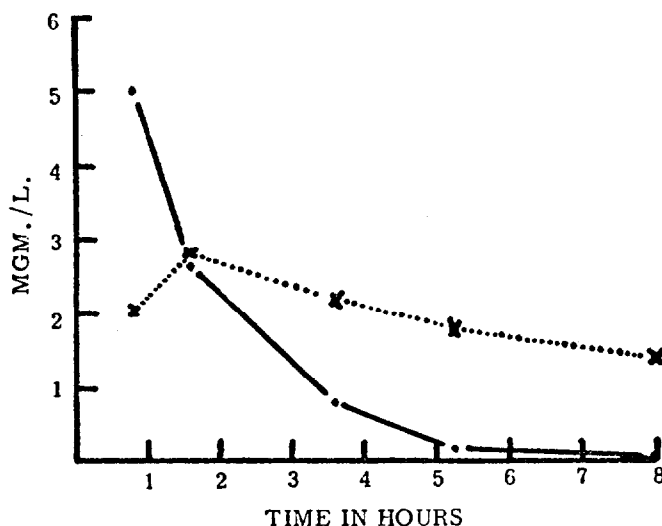


FIG. 3. Plasma levels of ephedrine (solid line) and norephedrine (dotted line) after the intravenous administration of 20 mgm. per kgm. of *l*-ephedrine hydrochloride to a dog.

The distribution of ephedrine and norephedrine was examined in representative tissues of two dogs which were given 50 mgm. per kgm. of *l*-ephedrine hydrochloride intraperitoneally. The animals were sacrificed by an intravenous injection of air one and two hours after the administration of the drug and the tissues sampled immediately afterwards. In dog 6, sacrificed one hour following drug administration, the tissue concentrations of ephedrine were higher than

those of norephedrine (table 3). Both compounds were localized in organ tissues to a considerable extent with negligible localization in body fat. The high concentration of the compounds in the brain and cerebrospinal fluid would suggest that there is little hindrance to their passage through the blood-brain barrier. The high concentration of ephedrine in the cerebrospinal fluid as compared to plasma is unusual and may be due to the relatively slow diffusion of the drug from cerebrospinal fluid as the drug rapidly disappears from plasma. In dog 7, sacrificed 2 hours following the administration of the drug, the concentration of norephedrine in tissues is considerably higher than the concentration of ephedrine indicating that most of the ephedrine had been demethylated within two hours.

TABLE 3

Distribution of ephedrine and norephedrine after the administration of ephedrine

Two dogs received 50 mgm./kgm. *l*-ephedrine intraperitoneally. Dog 6 was sacrificed one hour and Dog 7 was sacrificed two hours after the drug administration.

	DOG 6		DOG 7	
	Ephedrine mgm./kgm.	Norephedrine mgm./kgm.	Ephedrine mgm./kgm.	Norephedrine mgm./kgm.
Plasma.....	8.6	4.6	0.9	5.7
CSF.....	22	0	4.0	4.1
Liver.....	172	31	25	41
Lung.....	85	21	16	44
Kidney.....	108	8.5	28	77
Brain.....	127	5.5	18	29
Muscle.....	72	5.0	8.3	12
Heart.....	68	20	8.3	15
Spleen.....	103	9.3	15	40
Fat.....	6.8	0	11	4.4

Fate of the metabolites of ephedrine in the dog: The importance of norephedrine, an active pressor agent (Chen, Wu and Hendrickson, 1929), in the overall metabolic transformation of ephedrine, prompted a study of the fate of this compound in the dog. Two dogs (1 and 2) were given 20 mgm. per kgm. of *l*-norephedrine hydrochloride intravenously and the plasma levels and urinary excretion of the drug were measured. Plasma levels of norephedrine declined at a rate of about 25 per cent per hour (fig. 4), as compared with ephedrine which disappeared at a rate of 50 per cent per hour (fig. 3). About 72 per cent of the administered norephedrine was excreted unchanged, the remainder disappearing by an unknown route.

Only small amounts of hydroxylated ephedrine and norephedrine, both potent pressor agents (Chen, Wu and Hendrickson, 1929), were found in the urine but the possibility remained that these compounds may have been formed in considerable amounts and then further metabolized. To investigate this possibility, the fate of *p*-hydroxyephedrine and *p*-hydroxynorephedrine was examined in a dog. Dog 2 was given 15 mgm. per kgm. of *dl-p*-hydroxyephedrine hydrochloride intravenously, following which plasma levels of the drug and the urinary

excretion of the free and conjugated compound were measured at various intervals of time. The compound was found to disappear from the plasma at a rate of 45 per cent per hour. About 60 per cent of the administered drug was found in the urine as free and conjugated *p*-hydroxyephedrine.

Following the administration of *dl-p*-hydroxynorephedrine in the same dosage, the plasma levels declined about 40 per cent per hour. About 50 per cent of the administered drug was recovered in the urine as free and conjugated *p*-hydroxynorephedrine. These results indicate that both *p*-hydroxyephedrine and *p*-hydroxynorephedrine are relatively stable in the body. It may be concluded from these observations that hydroxylation is a relatively minor pathway in the metabolism of *l*-ephedrine in the dog.

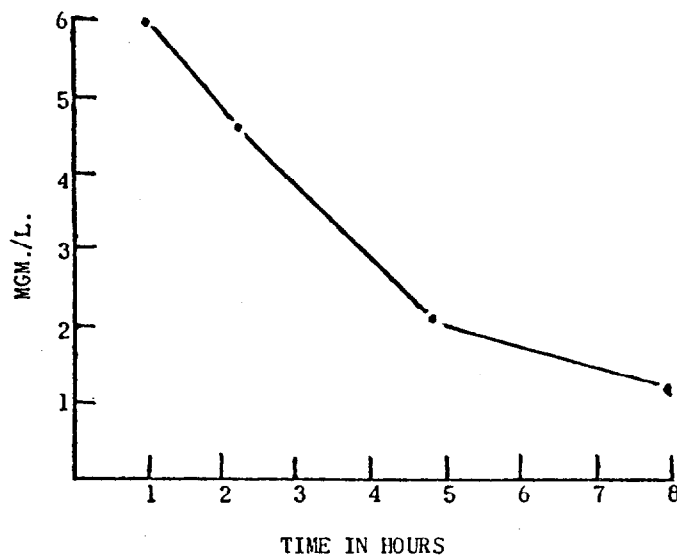


FIG. 4. Plasma levels of norephedrine after the intravenous administration of 20 mgm. per kgm. of *l*-norephedrine hydrochloride to a dog.

Fate of ephedrine in guinea pigs, rats and rabbits: The metabolic transformation of ephedrine was studied in guinea pigs, rats (Sprague-Dawley), and rabbits. Each animal was given 50 mgm. per kgm. of *l*-ephedrine hydrochloride intraperitoneally and the subsequent 24 hour urine sample was examined for ephedrine and metabolic products (table 4). Dogs and guinea pigs excreted only small amounts of ephedrine, considerable amounts of norephedrine, and a small amount of the hydroxylated derivatives. Rats, on the other hand, excreted relatively small amounts of norephedrine but considerable quantities of both ephedrine and its hydroxylated derivatives. These results suggest that the demethylation process is relatively slower in the rat so that hydroxylation and renal elimination of ephedrine are favored. The rabbit excreted only small amounts of ephedrine, norephedrine, and *p*-hydroxyephedrine.

To further delineate the intermediate metabolism of ephedrine in the rabbit,

the fates of norephedrine and *p*-hydroxyephedrine were examined in this species. After the administration of 10 mgm. per kgm. of *dl-p*-hydroxyephedrine to 2 rabbits, the major portion, about 65 per cent of the administered compound, was excreted in the free and conjugated form. When 25 mgm. per kgm. of *l*-norephedrine was administered to 2 rabbits only 3 per cent of the drug was excreted. These results suggest that in the rabbit ephedrine is demethylated to norephedrine which in turn is extensively metabolized.

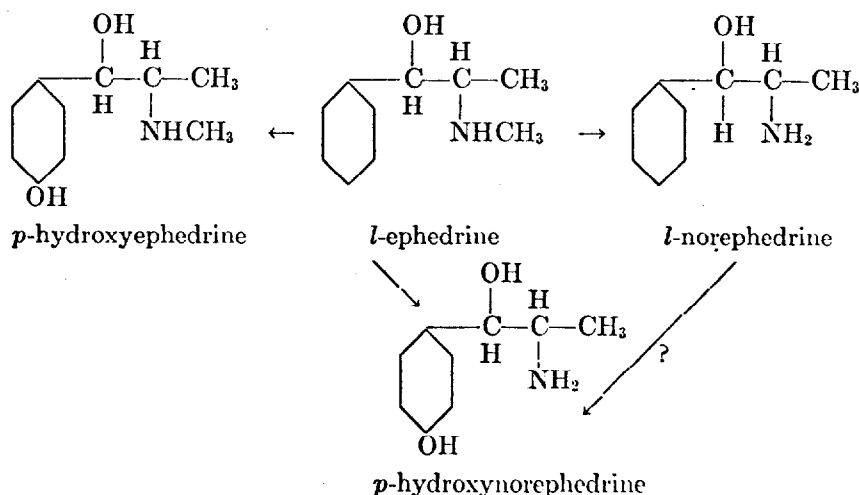
TABLE 4

Fate of ephedrine in a number of species

Fifty mgm./kgm. *l*-ephedrine were administered intraperitoneally to each animal. The urine was collected over a period of 24 hours. The proportion of the various metabolites is expressed in percentage of the amount of ephedrine administered.

SPECIES	NUMBER OF ANIMALS USED	EPHEDRINE	NOREPHEDRINE	TOTAL HYDROXY-EPHEDRINE AND HYDROXY-NOREPHEDRINE
		%	%	%
Dog.....	5	6.5	57.8	1.5
Guinea Pig.....	3	2.0	38.5	0.9
Rat.....	4	32.0	7.5	12.8
Rabbit.....	4	0.1	1.8	1.9

DISCUSSION. The following scheme for the route of metabolism of *l*-ephedrine in the dog is suggested by the studies described in this report.



The main route of metabolism involves rapid demethylation to *l*-norephedrine, a relatively stable and potent pressor agent. It may be concluded therefore that the activity of ephedrine is mediated to a considerable extent through norephedrine. Other, though minor, routes of metabolism involve hydroxylation of both ephedrine and norephedrine to yield the corresponding *p*-hydroxy derivatives,

both of which are also potent pressor agents. No evidence was found for the formation of 3,4-dihydroxyephedrine or 3,4-dihydroxynorephedrine.

Norephedrine is relatively stable in the dog and is excreted in the urine mainly unchanged. Calculations of renal excretion of norephedrine from plasma levels and urinary excretion of the drug yielded values which were considerably higher than the glomerular filtration rate, suggesting that a secretory transport mechanism is involved in its excretion.

Richter (1939) reported that in man ephedrine is excreted in the urine almost entirely unchanged and ascribed the stability of the drug *in vivo* to the failure of monoamine oxidase to catalyse the deamination of this compound. However, the procedure used by Richter for the estimation of ephedrine would not have distinguished between ephedrine and norephedrine. Previous work has shown that a number of alkylamines, including aminopyrine (Brodie and Axelrod, 1950), meperidine (Lief *et al.*, 1952) and mephobarbital (Butler, 1953), are demethylated in man; it seems likely that the same would hold true for ephedrine. However, at present it cannot be said whether the results obtained with dogs using large doses of ephedrine would carry over into man employing therapeutic doses of the drug. Studies on the fate of *l*-ephedrine in man are planned for a future investigation.

There are considerable differences in the metabolism of ephedrine by different species. In the dog and the guinea pig demethylation of the drug proceeds rapidly constituting a major route of biotransformation. The rat, on the other hand, demethylates ephedrine slowly and considerable amounts of the drug are excreted both unchanged and as hydroxylated derivatives. The rabbit excretes only negligible amounts of norephedrine but demethylation cannot be ruled out since norephedrine is almost completely metabolized in this animal.

SUMMARY

Methods for the estimation of ephedrine and its metabolic products, norephedrine, *p*-hydroxynorephedrine and *p*-hydroxyephedrine, in biological materials, are described.

In the dog the main route of biotransformation of ephedrine involves demethylation to norephedrine. The rate of demethylation is rapid, indicating that the activity of ephedrine is mediated largely through norephedrine. Norephedrine is excreted in the urine mainly unchanged. A minor route of metabolism of ephedrine involves hydroxylation of the aromatic nucleus to form *p*-hydroxyephedrine and *p*-hydroxynorephedrine, which are excreted partly free and partly as conjugates. Both ephedrine and norephedrine are highly localized in various organ tissues. Demethylation to norephedrine is a major route of metabolism in the dog and guinea pig and a minor pathway in the rat. On the other hand, hydroxylation of the drug constitutes a minor pathway of metabolism in the dog and guinea pig but a major one in the rat.

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REFERENCES

- BEYER, K. H.: *Physiol. Rev.*, **26**: 169, 1946.
BEYER, K. H., AND SHAPIRO, S. H.: *Am. J. Physiol.*, **144**: 321, 1945.
BRODIE, B. B., AND AXELROD, J.: *THIS JOURNAL*, **99**: 171, 1950.
BRODIE, B. B., AND UDENFRIEND, S.: *J. Biol. Chem.*, **158**: 705, 1945.
BRODIE, B. B., UDENFRIEND, S., AND BAER, J. J.: *J. Biol. Chem.*, **168**: 299, 1947.
BUTLER, T. C.: *THIS JOURNAL*, **106**: 235, 1952.
CHEN, K. K., WU, C. K., AND HENDRICKSON, W.: *THIS JOURNAL*, **36**: 363, 1929.
LIEF, P. A., BURNS, J. J., PAPPER, E. M., BERGER, B. L., WOLLACK, A., AND BRODIE, B. B.:
 Fed. Proc., **369**: 11, 1952.
RICHTER, D.: *Biochem. J.*, **32**: 1763, 1938.